Sorting Look-Alike Soybeans

Genetic fingerprinting aids plant variety protection.

A few years ago, breeders at Northrup King Co. produced a new soybean ideal for making tofu.

Called S16-60, the new variety had about 40 percent protein when dried—perfect for producing the fermented curd popular in vegetarian salads and cooked foods.

In field trials, S16-60 also yielded about 5 percent more soybeans than similar varieties bred for planting in the colder, northern Corn Belt states such as Minnesota, Wisconsin, South Dakota, and Michigan, says John Thorne. He is soybean research director for Northrup King in Washington, Iowa.

Before the company marketed S16-60 seed, however, Thorne wanted to protect the new variety from being copied by competing seed companies. To do that, he needed a plant variety protection certificate—similar to a patent for plants. So in 1994, he filed an application for one.

Thorne's application was sent to Jeffrey L. Strachan, a U.S. Department of Agriculture plant examiner who reviews such applications for soybeans and other plants. As part of his review, Strachan ran a routine check on his computer database to compare S16-60 with the 745 protected soybean varieties that had already received certificates, and with 1,500 others in the public domain.

The computer check compares the size, shape, and color of seeds, leaves, and flowers, as well as disease resistance and other traits. By comparing these and other characteristics, Strachan could determine if S16-60 was unique and deserved a certificate.

Strachan's check revealed, however, that S16-60 was virtually identical to another Northrup King soybean called B117, which had received a certificate in the 1980's and was a parent of S16-60. The discovery of a similar variety was bad news for Thorne.

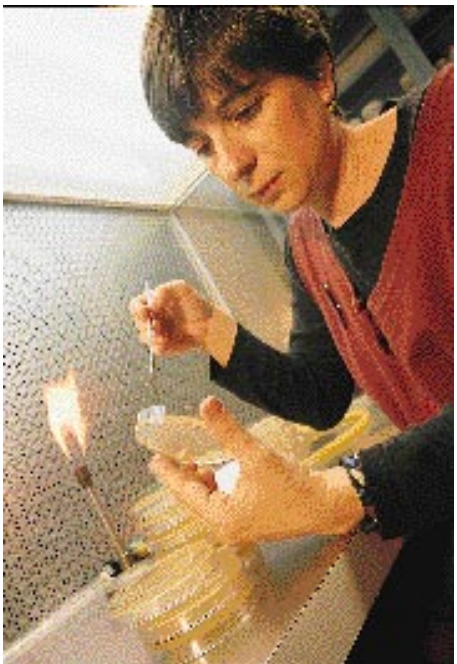
Because the two soybeans appeared so alike, the burden fell on Thorne to show specific differences between S16-60 and B117. In past years, this would have required extensive field plantings to collect and report additional field data to bolster his case.

"Those field tests are tedious and time consuming and subject to the vagaries of the weather," Thorne said. "They would probably have delayed the application for a year."

Enter Perry B. Cregan, a U.S. Department of Agriculture scientist at Beltsville, Maryland. Since 1991, Cregan, a plant geneticist with USDA's Agricultural Research Service, has been developing genetic maps for soybeans. Such maps are invaluable to plant breeders for creating new varieties of crops with specific traits such as disease resistance and oil and protein quality factors.

A byproduct of this research is that the same markers that help shape a genetic map can be used to create a genetic "fingerprint" for each variety.

Geneticist Noa Diwan transfers selected alfalfa DNA clones as part of the process of making genetic markers. (K7329-13)



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Even two plants that look identical can be differentiated by their genetic fingerprints.

Strachan, who works for USDA's Agricultural Marketing Service in Beltsville, had been working with Cregan on a cooperative project to develop a genetic fingerprinting system that can be transferred to private industry. After Strachan searched his database for groups of similar soybeans, Cregan obtained seed of 36 similar varieties from seed companies and state universities. Included among those seeds were S16-60 and B117.

Cregan then developed a unique fingerprint for each variety using genetic markers called simple sequence repeats (SSR's). These markers are repeating patterns of the four basic units of the genetic code that make up DNA, or deoxyribonucleic acid.

The four units, or nucleotides, are known by the initials A, G, C, and T—for adenine, guanine, cytosine, and thymine. An example of an SSR might be the sequence AT repeated 10, 15, or up to 40 or 50 times.

Cregan compared the SSR fingerprints from S16-60 and B117 and discovered that even though the two varieties share many similar traits, they are distinct.

"Since B117 is one of the parents of S16-60, it is not surprising that the two appear to be so similar," Cregan says. "But their DNA, or genetic code, did show several differences."

Soybean S16-60 is only one recent example of how genetic fingerprinting techniques are helping scientists sort out look-alike plants. Strachan says he expects to issue a certificate for the Northrup King soybean—and that the technology will help him in similar cases in the future.

"This is one of the most innovative leaps in technology, in terms of applied research, that we've seen in

the area of plant variety identification," Strachan says. "I'm seeing more and more applications where companies are submitting genetic profiles. By doing this, they're helping to move their applications through. And they're helping me construct a better database."

Strachan estimates that about 20 companies have submitted genetic profiles for roughly 100 different varieties. The companies are either developing the profiles in-house or contracting the job to outside biotech companies.

Cregan has completed about 100 genetic profiles for soybeans and is working on fingerprinting other crops such as alfalfa and wheat. He's also collaborating with scientists working with avocados and mangos. Strachan says the new technology will help identify varieties of alfalfa, corn, and small grains.

The technology is emerging just as certificates are becoming more popular as a way for companies to protect their breeding work. Certificates are issued under the Plant Variety Protection Act of 1970 (PVPA). Under the original act, a certificate was good for 18 years; 1994 amendments enable protection for 20 years.

If a company has a certificate on a variety, other companies must get permission to sell that variety. The company that receives the certificate agrees to disclose to the public basic information about its variety, such as a description of the plant and its breeding history. For each plant that receives a certificate, 2,500 seeds are sent to the ARS National Seed Storage Laboratory, a genetic repository in Fort Collins, Colorado, where they will be preserved.

Since PVPA was enacted, the AMS Plant Variety Protection Office at the National Agricultural Library has received 4,908 applications and issued 3,600 certificates for more than 100 plant species. These include soybeans (745), corn (352), wheat (354), beans (303), peas (254), and cotton (221). As of January 1996, more than 750 applications were pending with Strachan and other examiners at the office.

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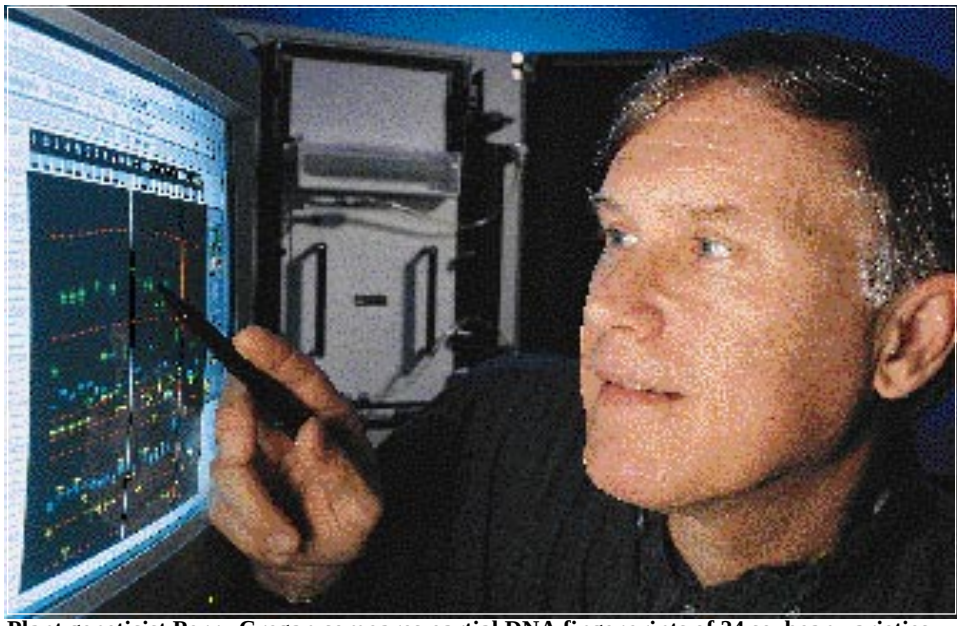

Plant geneticist Perry Cregan compares partial DNA fingerprints of 24 soybean varieties. (K7327-8)

Strachan also notes that companies are using the new technology to resolve disputes in court. A company that thinks its rights have been violated can file a civil suit against anyone it believes has infringed on the certificate. "This technology will become a very useful tool in such cases," said Strachan.

Overall, Cregan's fingerprinting research is part of a larger collaborative soybean mapping effort with scientists at the Universities of Nebraska and Utah, USDA-ARS at Iowa State University, and a biotech company called BioGenetics Services, Inc., of Brookings, South Dakota. The USDA Plant Genome Program, National Research Initiative Competitive Grants Program, and United Soybean Board have assisted with funding.

Cregan says Research Genetics of Huntsville, Alabama; BioGenetics Services; and Linkage Genetics, based in Salt Lake City, Utah, may be interested in commercializing the technology.

"We hope to develop a set of more than 600 soybean SSR DNA markers," Cregan says. "These can be used for many purposes—including developing a genetic map for soybeans. This would help breeders find genes related to disease resistance, oil and protein content, and other valuable traits."—By **Sean Adams,** ARS.

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